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The Prostate 38:73–78 (1999)

Rapid Communication

Infusion of Dendritic Cells Pulsed With HLA-A2-Specific Prostate-Specific Membrane Antigen Peptides: A Phase II Prostate Cancer Vaccine Trial Involving Patients With Hormone-Refractory Metastatic Disease

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BACKGROUND. A phase II trial was conducted to assess the efficacy of infusions of dendritic cells (DC) and two HLA-A2-specific PSMA peptides (PSM-P1 and -P2). This report describes thirty three subjects with hormone-refractory metastatic prostate cancer without prior vaccine therapy history who were evaluated and reported as a group.

METHODS. All subjects received six infusions of DC pulsed with PSM-P1 and -P2 at six week intervals. Clinical monitoring was conducted pre-, during, and post- phase II study. Data collected include: complete blood count, bone and total alkaline phosphatase, prostate markers, physical examination, performance status, bone scan, ProstaScint® scan, chest x-ray, as well as assays to monitor cellular immune responses.

RESULTS. Six partial and two complete responders were identified in the phase II study based on NPCP criteria, plus 50% reduction of prostate-specific antigen (PSA), or resolution in previously measurable lesions on ProstaScint® scan.

CONCLUSIONS. Over 30% of study participants in this group showed a positive response at the conclusion of the trial. This study suggested that DC-based cancer vaccines may provide an alternative therapy for prostate cancer patients whose disease no longer responds to hormone therapy. *Prostate 38:73–78, 1999.* © 1999 Wiley-Liss, Inc.

KEY WORDS: **dendritic cells (DC); prostate-specific membrane antigen (PSMA); immunotherapy; cancer vaccine; hormone-refractory; metastatic; prostate cancer; clinical trial**

INTRODUCTION

The most recent advances in cancer vaccines have included the use of autologous antigen-presenting cells (APC) to present cancer antigens to patient T cells [1]. The rationale of utilizing these specialized cells is to let APC provide all factors necessary for initiation of the immune response, including those that are not

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yet defined [1,2]. Dendritic cells (DC) are considered the most potent APC of the immune system [2]. Our approach to a prostate cancer vaccine utilizes cultured patient DC and prostate-specific membrane antigen (PSMA) to activate prostate cancer-specific autologous T cells in vivo [3]. Our initial phase I trial demonstrated that infusions of autologous DC and HLA-A2-specific PSMA peptides were well tolerated by all 51 study participants with hormone refractory advanced prostate cancer. In addition, favorable cellular immune responses and significant decreases in prostate-specific antigen (PSA) level were observed in seven partial responders, identified based on NPCP criteria, and a 50% reduction of PSA level [4,5]. Our phase II clinical trial enrolled a total of 107 subjects who received six infusions of DC pulsed with two HLA-A2-specific PSMA peptides. Sixty-six subjects were admitted with hormone refractory metastatic prostate cancer. All patients in this group who were treated with hormonal therapy before the start of the trial, remained on the same therapy throughout this trial. If an anti-androgen had been stopped, no patient was entered until after 3 months.

Half of the hormone-refractory metastatic group (33/66) were participants in the previous phase I study, who had requested to be enrolled in the phase II study. We have previously reported that nine partial responders were identified from this group based on NPCP criteria, and a 50% reduction of PSA level [6]. Four of the partial responders were also responders in the phase I study. Their average response period was over 370 days. Five other responders were non-responders in the phase I study. Their average response period was 196 days at the time of the report. This paper will describe the evaluation of the other half of this group: hormone refractory metastatic subjects with no previous immunotherapy experience.

MATERIALS AND METHODS

Reagents and Cytokines

PSMA Peptides with HLA-A0201-specific motif (PSM-P1: LLHETDSAV and PSM-P2: ALFDIESKV) were synthesized and purified (>95% purity) by Peninsula Laboratories, Inc. (Belmont, CA), and obtained as a lyophilized powder. The powder was dissolved in 0.9% saline (USP 0.9%, sodium chloride injection, American Reagent Laboratories, Shirley, NY) to a concentration of 2 mg/ml. The peptide solution was sterilized using 0.2 μm filtration. Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) approved for human use was provided by Immunex Corp. (Seattle, WA). Interleukin-4 (IL-4) was purchased from Peprotech, Inc. (Rocky Hill, NJ).

DC Culture

Each participant was subjected to leukapheresis at the Fred Hutchinsons Cancer Research Center (or alternatively blood draws) prior to the start of the trial. DC were cultured as described previously [5]. In short, peripheral blood mononuclear cells (PBMC) were isolated using Histopaque 1077 Ficoll (Sigma Chemical Co., St. Louis, MO) density gradient. PBMC were resuspended in complete medium (OPTIMEM medium [GIBCO-BRL, Grand Island, NY] and 5% heat-inactivated autologous plasma) and plated in a 75 cm^2 tissue culture flask ($2–3 \times 10^7$ cells/flask). Cell suspensions were incubated in a humidified incubator (37°C, 5% CO_2) for 60 minutes. Non-adherent cells were removed and adherent cells were washed gently with warm (37°C) complete medium. DC propagation medium (DCPM: complete medium, 500 units/ml GM-CSF and 500 units/ml IL-4) was added to the adherent cells (10 ml/flask). These cells were cultured for 7 days.

Administration of DC Pulsed With PSMA Peptides

Cultured DC were incubated for 2 hours in the presence of 10 μg/ml PSM-P1 and -P2 peptides, washed, resuspended in 10 ml injection grade saline, and delivered to Northwest Hospital Day Surgery/Short Stay unit. The DC suspension was infused over 30 minutes with a total volume of 100 ml 0.9% saline. Six infusions of autologous DC pulsed PSM-P1 and -P2 peptides were administered at six week intervals. All study participants subjected to clinical monitoring as described.

Clinical Monitoring

Patients were followed before, during and after treatment with periodic PSA (prostate specific antigen; Tandem-E PSA kit, Hybritech Incorporated, San Diego, CA), free PSA (Tandem-R PSA kit, Hybritech Incorporated), PSMA (in-house western blot assay), complete blood counts, CHEM-22, bone alkaline phosphatase (Tandem-R Ostase kit, Hybritech Incorporated), inital chest x-ray, bone scans and ProstaScint® scans [7,8]. All testing was conducted on an outpatient basis at Northwest Hospital. A delayed-type hypersensitivity (DTH) test to measure patients general immune response activity were conducted at the beginning of the phase I study and repeated during the phase II study. Recall antigens tested were: tetanus, diphtheria, streptococcus, tuberculin, glycerin, candida, trychophyton, and proteus. Patients were also evaluated every infusion cycle by one of the study physicians.

TABLE I. Study Population Summary (n = 25)*

Age	
Mean:	64
Median:	63
Range:	43 to 82
HLA-A2	
Positive:	14
Negative:	11
Prostate Cancer Stage	
D1 (T4N1-3M0)	2/25 (8%)
D2 (T4N1-3M1a-c)	23/25 (92%)
Original Therapy	
Hormone Therapy:	13
Radical Prostatectomy:	7
Radiation Therapy:	5
Current Hormone Therapy	
LHRH:	5
LHRH + Anti-Androgen:	14
Orchiectomy:	2
Orchiectomy + Anti-Androgen:	3
Estrogens:	1

*Out of 33 subjects who were initially enrolled in the study, 25 participants received at least one infusion.

RESULTS

Phase II Trial: Study Population

Thirty-three patients with hormone-refractory metastatic prostate were initially enrolled. All patients remained on the same hormone therapy throughout this trial. If an anti-androgen had been stopped, no patient was entered until after 3 months. Twenty-five subjects completed at least one infusion cycle, and were considered evaluable. Their clinical staging, HLA-A2 status, and prostate cancer therapy history are summarized in Table I. Six infusions of DC pulsed with PSM-P1 and -P2 peptides were administered at six week intervals. The number of DC infused (average = 17 million; range = 5–24 million) varied with each patient, based on the number of cells obtained from each culture.

Twenty-five study participants were evaluated for response, based on the National Prostate Cancer Project (NPCP) criteria, plus a 50% decrease in PSA, or significant improvements in repeat ProstaScint® scan. Six subjects (24%) were identified as partial responders, two (8%) as complete responders. Among the non-responders, 16 subjects (64%) exhibited disease progression, and one (4%) showed no significant change in disease state (Fig. 1). Among those evaluated with disease progression, only 7 subjects (44%) completed the study. The other 7 subjects (44%) died during the study period, and two (13%) withdrew from the study.

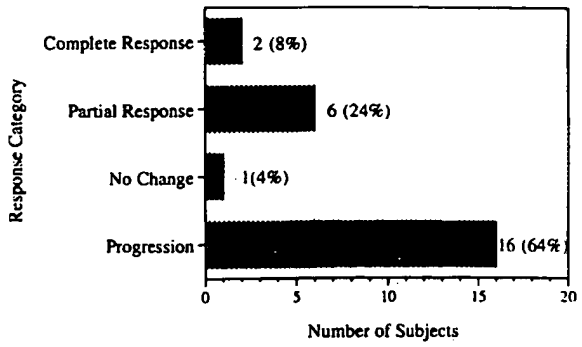

Fig. 1. Clinical evaluation of twenty-five subjects in The Phase II Clinical Trial. The number and percentage of subjects in four clinical response categories to the therapy are shown.

In addition to clinical monitoring, each patient was also subjected to delayed-type hypersensitivity (DTH) skin testing to measure the general immune response activity. These tests were conducted before, and after the study. In the responder group, 6/8 subjects exhibited no change, and 2/8 showed a decrease in skin reactivity. In the non-responder group, 11 subjects did not receive their final skin tests. Among the 6 patients who completed the study, 1/6 subjects exhibited no change, 1/6 subjects showed increased, and 4/6 showed decreased skin reactivity. The average post-study Karnofsky scores for the responder and non-responder groups were 90 and 80, respectively.

Profiles of Responders in The Phase II Study

Eight subjects (32%) in this group showed significant improvements detected by the clinical evaluations conducted both during the study and after completion of all six infusions. These subjects were evaluated for an average of 335 days, which included 6 infusion cycles and a follow-up observation period. The profiles of these eight responders, which include their study entry disease stage, HLA-A2 status, and hormonal therapy are summarized in Table II. Two subjects were identified as complete responders (CR), and six others as partial responders (PR). Three subjects showed at least 50% decline in their serum PSA level with maintenance of the decline on at least three separate determinations spaced at least two weeks apart (subjects A, D, and H). The remaining five responders started the study and have maintained a baseline level PSA throughout the study period. One of these subjects demonstrated resolution (subject B) and another (subject F) showed significant regression of lymph node lesions, as detected by post-study ProstaScint® scans and prostate biopsy. Three subjects showed significant improvements (subjects D, E, and G) and one subject (subject C) showed resolution of

TABLE II. Eight Responders Identified From 25 Study Participants in The Phase II Clinical Trial*

Subject	Stage	HLA-A2	Current hormonal therapy	Improvement in PSA level	Improvement in Prosta Scint	Improvement in Bone scan	Clinical response
A	D2	+	Zoladex, Dexamethosone	yes	negative	negative	PR
B	D2	+	Lupron + Casodex	bsl	yes	negative	CR
C	D2	–	Lupron	bsl	negative	yes	CR
D	D2	–	Estrogens	yes	NE	yes	PR
E	D2	+	Leupron + Eulexin	bsl	NE	yes	PR
F	D2	+	Lupron + Casodex	bsl	yes	negative	PR
G	D2	+	Orchiectomy + Casodex	bsl	NC	yes	PR
H	D2	–	Lupron + Casodex	yes	NC	no	PR

*bsl, baseline level; NE, ProstaScint not evaluable due to human-anti mouse antibody (HAMA) reaction; NC, no significant change; CR, complete response; PR, partial response.

metastatic lesions of the bone, as detected by post-study bone scans.

Figure 2 depicts PSA levels of subjects A, and H throughout the study and observation period. Subject A entered the study with a history of supraclavicular lymphadenopathy. Staining of the biopsy specimen with antibodies to PSA, PSMA and prostatic acid phosphatase (PAP) confirmed the prostatic origin. The PSA levels stayed around 4 ng/ml through the first 4 infusion cycles. A decline in PSA level was detected starting at infusion 5 and the level has remained at 1.5 ng/ml for more than 6 weeks. Subject H was enrolled in the study with extensive skeletal metastases detected by a pre-study bone scan showing heterogeneous uptake in all the visualized bones, most prominent in the thoracic spine and calvarium, extending into the knees and humeral shafts. In addition, the pre-study ProstaScint® scan showed multifocal pelvic and abdominal lymph node metastases. The patient entered the study with a PSA level of over 700 ng/ml, which continued to rise to a high of 3105 ng/ml during the first two infusion cycle. A partial response was apparent following a sharp PSA decrease observed between infusions 2 and 3. This PSA decline continued for over 100 days to a low level of 250 ng/ml. After infusion 5, the PSA level increased again and remained at around 800 ng/ml.

Subject C entered the study with a history of extensive skeletal metastases, and a Gleason 8 prostate biopsy. He had undergone chemotherapy prior to enrollment in this study. The pre-study bone scan already showed some overall improvement, although there were 9 persistent foci of increased bone uptake, which included the upper mid cervical spine, right mid thoracic spine, pelvis, and the proximal femurs. The initial ProstaScint® scan after the start of therapy showed no nodal lesions. The PSA level stayed at a baseline level throughout the trial. At the conclusion of the study, the repeat ProstaScint® scan was negative, and repeat bone scan was negative (Fig. 3). Two different repeat prostate biopsies conducted at two different institutions showed no prostatic tumor was present. Another complete responder (subject B) had disppearance of nodal disease, and a negative prostate biopsy (previously T2b, Gleason 6). Both complete responders had no therapy specifically directed to the prostate at any time.

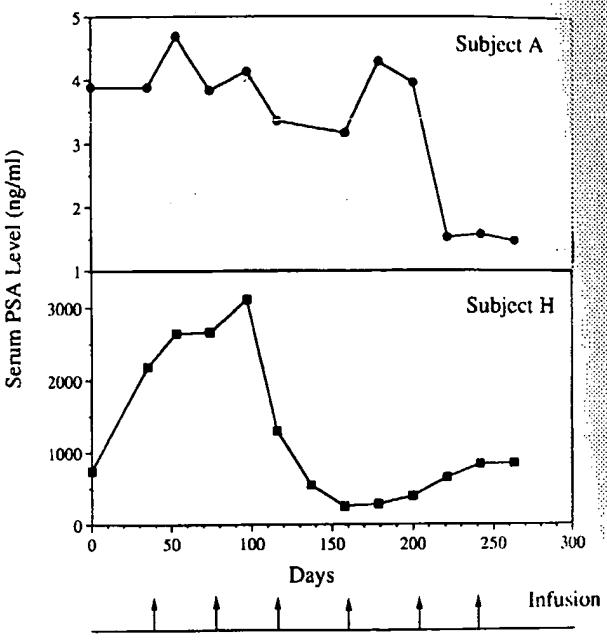

Fig. 2. Decrease of prostate-specific antigen levels in two partial responders. Serum PSA levels were measured on the day of each infusion and three weeks after that throughout the trial. The corresponding infusions are indicated by arrows.

DISCUSSION

Current standard prostate cancer treatments for early-stage, localized prostate cancers, e.g., radical

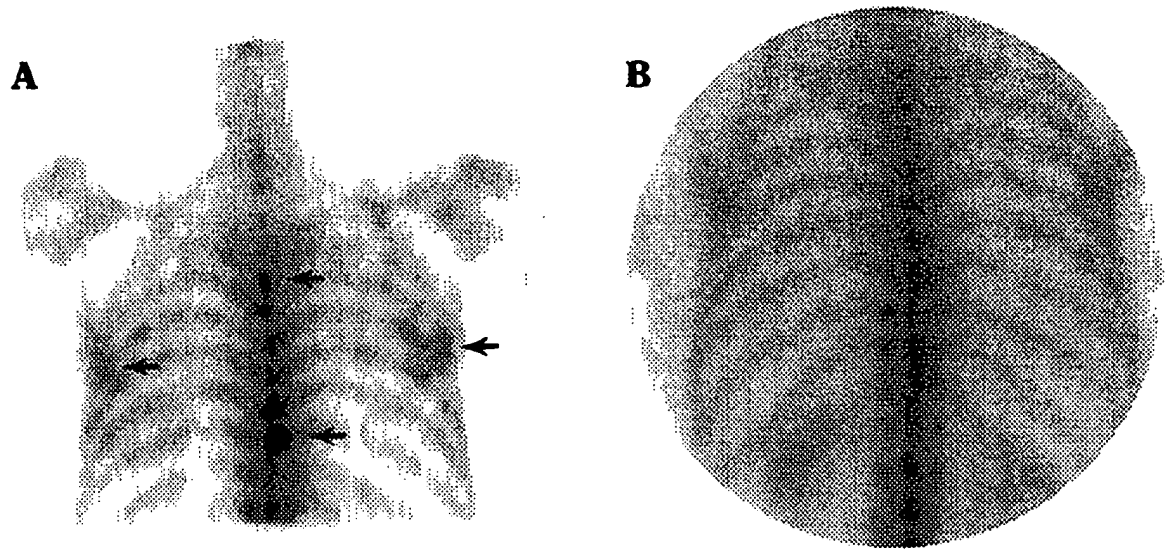

Fig. 3. Comparison of bone scans of a complete responder taken before and after the study. **A:** Skeletal metastatic lesions (indicated by arrows) were detected by a bone scan taken at the beginning of the study. **B:** A repeat bone scan conducted after the completion of 6 infusion cycles showed resolution of these lesions.

prostatectomy or radiation therapy, exhibit failure rates of more than 20% [9]. As a result, there has been an increasing number of patients who subsequently either manifest metastatic disease or who are at high risk for the development of such a state. The options for these primary treatment failures, which include hormonal, chemotherapeutic, or radiation strategies, are limited in terms of duration and efficacy [10]. This report describes the clinical evaluation of a group of 25 subjects with hormone refractory metastatic prostate cancer, who participated in a phase II clinical trial involving 6 administrations of autologous DC pulsed with two HLA-A2-specific PSMA peptides. Thirty-two percent of the study population (8/25) showed significant clinical response to the therapy. The average total evaluation period was 335 days. Six subjects were partial responders, and two subjects were complete responders. This study suggested that DC-based cancer vaccines may provide an alternative therapy for prostate cancer patients whose disease no longer responds to hormone therapy.

Although a majority of the responder population (5/8; 63%) express HLA-A2, a number of clinical responder are HLA-A2 negative (3/8; 37%). Possible explanations for these results include the ability of DC to capture and present other antigens after being infused back into the patients, and the affinity of PSM-P1 and -P2 to other HLA molecules. Comparisons of pre-and post- DTH skin testing using recall antigens suggested some correlation between the state of general immune response and clinical response. Among the responder groups, a majority of the population (6/8) maintained the state of their immune reactivity, while only 2/8 showed a decrease. In contrast, 4/6 non-responders who received repeat DTH tests showed a reduction in skin test reactivity, while only 2/6 maintained or improved in their general immune reactivity. Other studies to monitor specific T cell mediated immune responses, including T-cell lymphoproliferative assays, interferon-γ and interleukin-10 secretions upon presentation of PSMA peptides in vitro, and specific cytotoxic responses against a panel of prostate cancer lines are still being conducted.

Evaluations of the other study population who participated in this phase II clinical trial, i. e., 41 subjects with locally advanced recurrently prostate cancer, are currently being conducted and will be reported separately. In our next clinical trial, we will expand the antigen repertoire of our vaccine study from two PSMA peptides (each of which represents 9 amino acids) to a recombinant PSMA protein consisting of the native sequence without the transmembrane domain. This change will allow for participations of multiple HLA/peptide combinations as the prostate cancer vaccine.

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